

Plant reproductive traits mediate tritrophic feedback effects within an obligate brood-site pollination mutualism

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Received: 30 March 2015 / Accepted: 5 June 2015
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Abstract Plants, herbivores and parasitoids affect each other directly and indirectly; however, feedback effects mediated by host plant traits have rarely been demonstrated in these tritrophic interactions. Brood-site pollination mutualisms (e.g. those involving figs and fig wasps) represent specialised tritrophic communities where the progeny of mutualistic pollinators and of non-mutualistic gallers (both herbivores) together with that of their parasitoids develop within enclosed inflorescences called syconia (hence termed brood-sites or microcosms). Plant reproductive phenology (which affects temporal brood-site availability) and inflorescence size (representing brood-site size) are plant traits that could affect reproductive resources, and hence relationships between trees, pollinators and non-pollinating wasps. Analysing wasp and seed contents of syconia, we examined direct, indirect, trophic and non-trophic

relationships within the interaction web of the fig–fig wasp community of *Ficus racemosa* in the context of brood site size and availability. We demonstrate that in addition to direct resource competition and predator–prey (host–parasitoid) interactions, these communities display exploitative or apparent competition and trait-mediated indirect interactions. Inflorescence size and plant reproductive phenology impacted plant–herbivore and plant–parasitoid associations. These plant traits also influenced herbivore–herbivore and herbivore–parasitoid relationships via indirect effects. Most importantly, we found a reciprocal effect between within-tree reproductive asynchrony and fig wasp progeny abundances per syconium that drives a positive feedback cycle within the system. The impact of a multi-trophic feedback cycle within a community built around a mutualistic core highlights the need for a holistic view of plant–herbivore–parasitoid interactions in the community ecology of mutualisms.

Communicated by Caroline Müller.

Electronic supplementary material The online version of this article (doi:10.1007/s00442-015-3372-9) contains supplementary material, which is available to authorized users.

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Keywords Inflorescence size · Interaction web · Feedback cycle · Plant–herbivore–parasitoid interactions · Trait-mediated indirect effect

Introduction

Tritrophic interactions involving plants are complex because variation in plant traits such as chemistry or phenology can modulate direct and indirect interactions within and between higher trophic levels (Ohgushi 2005; Poelman and Dicke 2014); e.g. predator–prey relationships are influenced by plant effects on prey detectability or palatability (Price et al. 1980; Ode 2006; Pagès et al. 2012). In a tritrophic network, interaction chains can lead to negative or positive indirect effects among organisms within the same

trophic level; negative effects may occur through exploitative (Colwell and Fuentes 1975; Wootton 1994) or apparent competition (Holt 1977; Wootton 1994). Positive interactions can also occur within or across trophic levels via indirect facilitation (Callaway 2007). Apart from direct and indirect interactions, interactions among community members can also include reciprocal effects leading to positive or negative feedback loops or cycles. Although positive or negative feedback loops are deemed important in tritrophic systems (Craig 2010; Peterson et al. 2013), they have rarely been investigated especially in the context of plant–insect mutualisms (Savage and Peterson 2007).

Mutualism-centred communities with obligate interactants and bounded membership, e.g. the brood-site pollination mutualisms of the yucca–yucca moth or fig–fig wasp systems, offer excellent models that allow examination of direct effects as well as indirect and feedback effects centred around plant traits. In these communities, the inflorescence or fruit is a nursery for the progeny of mutualistic pollinators and associated satellite wasp species parasitic on the mutualism (Pellmyr et al. 1996; Cook and Segar 2010). Since ovules are the definitive resource on which the entire community depends, plant traits affecting ovule availability will not only influence relationships between the plant and its insect nursery inhabitants but will also mediate indirect non-trophic relationships between the nursery occupants. Therefore, in these systems, within-plant reproductive phenology and inflorescence size are important factors that could affect relationships over several trophic levels.

We therefore examined the effects of reproductive phenology and inflorescence size on multitrophic interactions within the fig–fig wasp system. This is a closed system centered around an obligate pollination mutualism which also hosts several obligate non-mutualistic satellite species (Cook and Rasplus 2003; Cook and Segar 2010). Since these systems have restricted membership, reasoned predictions can be made on trophic and non-trophic interactions within them. A crop of fig syconia on a tree could be considered a collective of unitary resource patches or microcosms represented by individual syconia, with syconium volume as an indicator of resource patch size. These syconia, which can vary considerably in size, developing wasp and seed frequency even within a single reproductive episode on a tree, can also be considered as ‘independent reproductive units’, with the development of individual syconia being completed or interrupted based on their occupants (Jandér et al. 2012; Krishnan and Borges 2014). Therefore, individual syconia can be considered as independent replicative units to test for multiple interactions among syconium inhabitants and plant traits such as syconium volume and reproductive phenology. Furthermore, the system also offers a complex feedback cycle where plant

reproductive phenology and the abundance of fig wasps within syconia reciprocally affect each other (Krishnan et al. 2014). Variation in within-tree reproductive phenology could cause differential visitation of syconia by pollinators and non-pollinators; this leads to intra-tree variation in syconium inhabitants, which in turn results in varied developmental times of these syconia affecting within-tree reproductive synchrony (Krishnan and Borges 2014). Since there is convergence in community structure of fig wasps associated with individual fig species across continents (Segar et al. 2013), our findings will have general applicability and are likely to enrich other investigations.

We used the identities and the frequency of the different occupants of individual syconia (wasps and seeds) as replicate samples of communities, and the individual fig tree on which the syconia develop as the basal community substrate. We made a priori predictions on the direct, indirect and feedback relationships that exist between the lowest trophic level, i.e. the fig tree, and the abundances of higher trophic levels, i.e. the fig wasp fauna, within syconia, and examined their validity. For this, we investigated relationships between plant reproductive traits such as syconium volume and within-tree reproductive phenology with seed and fig wasp reproduction within syconia. Apart from the aforementioned plant reproductive traits, it is also possible that other traits such as the chemical composition and quality of syconia can influence seed and fig wasp reproduction. However, it was beyond the scope of this study to include such factors in our analysis. The effects of plant phenology on syconium contents and the feedback cycle between phenology and syconium contents are likely dependent on complex processes and could not be easily predicted a priori based on our current understanding of the system. Within trophic levels, we examined the direct and indirect effects of different groups of fig wasps (gallers or parasitoids) on each other’s abundance, and the role of plant reproductive traits in modifying these effects. Based on the patterns observed, we deduce possible effects of plant reproductive traits and non-pollinating fig wasps on the mutualism between the tree and its pollinator wasp. Our work therefore advances the general understanding of mutualism-centred communities and their organisation by exploring direct, indirect, trophic and non-trophic links in the interaction web.

Materials and methods

Species biology

Ficus racemosa (subgenus *Sycomorus*) is a monoecious fig distributed throughout the Indo-Australasian region. Trees produce 2–7 fig crops aseasonally every year with

reproduction occurring in enclosed inflorescences (figs or syconia) borne on tree trunks. Syconium development is divided into 5 phases (Galil and Eisikowitch 1968) outlined in Fig. 1. The syconia are pollinated by the mutualistic agaonid wasp *Ceratosolen fusciceps* Mayr and are also host to six other species of non-pollinating fig wasps in the agaonid subfamily Sycophaginae and the pteromalid subfamily Sycoryctinae (gallers: *Sycophaga stratheni* Joseph, *Sycophaga testacea* Mayr, *Sycophaga fusca* Girault; and the parasitoids: *Sycophaga agragensis* Joseph, *Apocrypta westwoodi* Grandi and *Apocrypta* sp. 2) that develop within them. The oviposition periods of each of these wasps during syconium development (Fig. 1b) were previously determined (Ranganathan et al. 2010). It should be noted that *Apocryptophagus* is synonymised with *Sycophaga* (Cruaud et al. 2011) and that *Apocrypta* is now placed in the Pteromalidae (Segar et al. 2012).

Trophic and non-trophic relationships in the system

A representation of the oviposition method and trophic levels of the various fig wasps is provided in Fig. 1a, c, and highlights trophic relationships likely to occur within the community. Since the galler *S. stratheni* (G0) is extremely rare, it was excluded from the analyses. Since pollinators oviposit from within the syconium, while non-pollinating gallers oviposit from outside (Fig. 1a), interference competition between pollinators and non-pollinating gallers is unlikely, although they compete for the same resource (ovules). Since galler G1 (*S. testacea*) oviposits before pollinator arrival (Fig. 1b), and G2 (*S. fusca*) oviposits slightly before and concurrent with the pollinator (Fig. 1b), they are likely to affect pollinator reproduction (defined as the number of pollinator offspring produced per syconium) indirectly through exploitative competition (Fig. 1c). Predator–prey (host–parasite) relationships can be expected between the parasitoids (P1 + P2 + P3) and the herbivorous pollinators and gallers (Fig. 1c) as the parasitoids prey upon (parasitise) galler and pollinator larvae (Fig. 1a). The external surfaces of *F. racemosa* syconia are often crowded with many ovipositing wasps (Fig. 2) which could also interfere with wasp reproduction due to intense intra-specific competition for oviposition space and resources.

Data collection

The initiation and development of syconia of 15 *F. racemosa* trees (20 bunches of marked syconia per tree) within the campus of the Indian Institute of Science (12°58'N, 77°35'E), Bangalore, India, was recorded between January and August 2009 every 2–3 days. For every flowering episode, a modified form of Augspurger's index of synchrony (Augspurger 1983) was calculated for within-tree

synchrony of the syconium phases; this index ranges from 0 (complete synchrony) to 1 (complete asynchrony) (Krishnan and Borges 2014).

Syconium occupants of 361 D-phase (wasp dispersal phase during which wasps and seeds are fully developed) syconia from 15 trees were sorted according to trophic levels into seeds (trophic level I), pollinators (trophic level II, mutualist), gallers (G1 + G2) (trophic level II, non-mutualist), parasitoids (P1 + P2 + P3) (trophic level III), and their within-syconium frequency determined. Syconium sizes (volumes) of the D-phase figs were determined as in Krishnan and Borges (2014).

Predictions about species interactions

All mention of fig wasps in the data analyses refers to the numbers of fully developed and eclosed offspring of the different species. We used path analysis (see justification later) to investigate species interactions. The a priori path model was constructed based on relationships derived from the known biology of the fig wasp fauna. A schematic representation of the possible relationship network and the a priori path model is outlined in Fig. 3 and Appendix A. For ease of presentation, the a priori predictions in Fig. 3 are segregated and represented in two parts adjacent to each other (Fig. 3a) according to whether they addressed the relationships between (1) the tree and fig wasp fauna, (2) feedback between the tree and fig wasp fauna, or (3) between the different groups of fig wasp fauna.

Interactions of trophic level I (the plant) with trophic levels II (herbivores) and III (parasitoids)

Syconium volume could be expected to have direct and positive effects on the abundances of all syconium inhabitants (Fig. 3a), since for most inhabitants the principal resource within the syconium is the number of female flowers (ovules) which is strongly correlated with syconium volume at D-phase (Krishnan and Borges 2014). Since within-tree asynchrony essentially affects the temporal availability of resources, it was expected to affect the reproduction of pollinators, gallers (G1 + G2) and parasitoids (P1 + P2 + P3). However, the direction of this effect could not be easily predicted (hence depicted by a question mark '?') as it could depend on various other factors such as the wasps' sensitivity to host location cues, oviposition period and intra- and inter-species competition (Fig. 3a). Low within-tree asynchrony provides many syconia for a short period of time (high resource availability over a short temporal period), whereas high within-plant asynchrony would offer fewer resources, but for a longer period of time. The relationship between pollinator progeny and seed numbers produced could be positive or negative (Fig. 3a) since

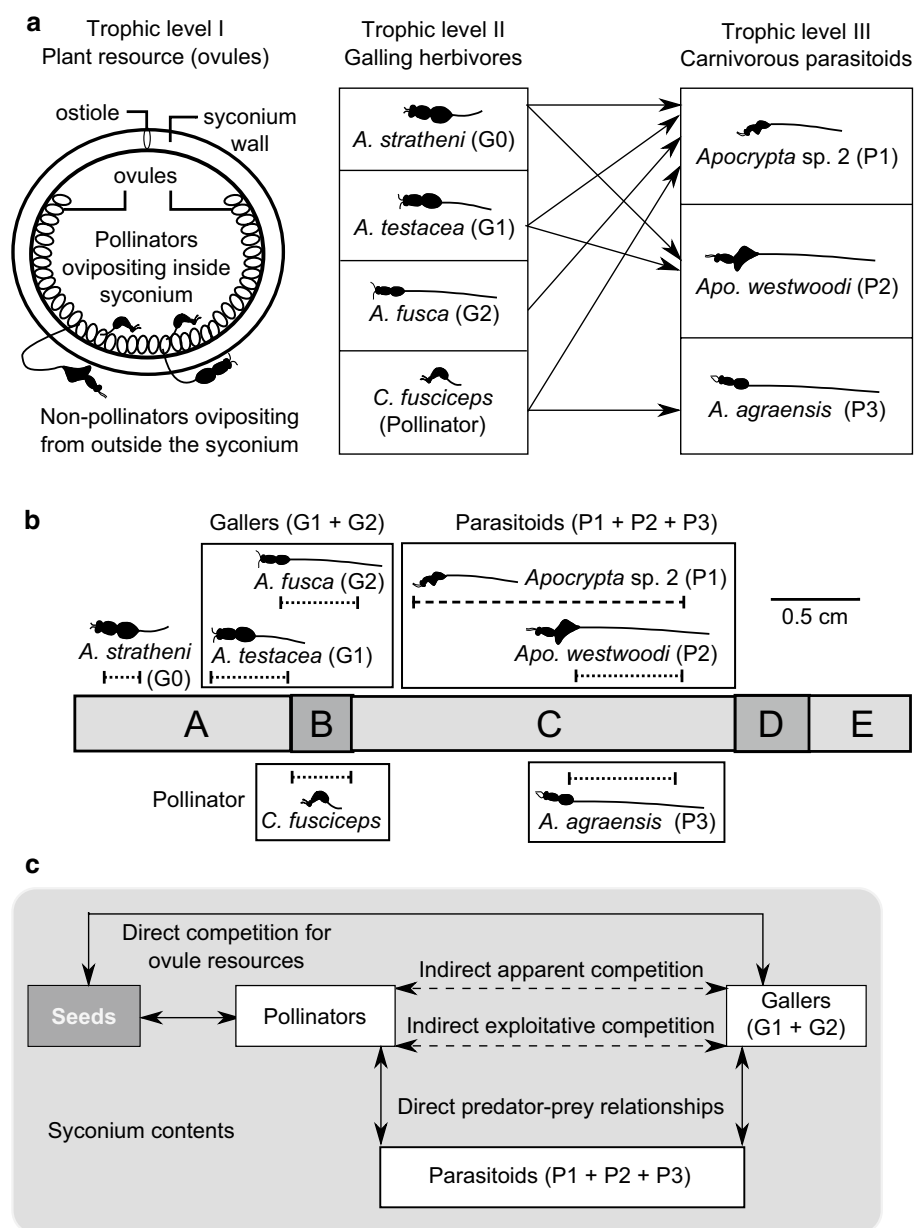


Fig. 1 Basic biology of *Ficus racemosa* fig wasp fauna with respect to oviposition periods, larval diets and expected relationships between various members of the community. **a** Schematic diagram of oviposition habits and food web indicating the trophic levels occupied by *F. racemosa* fig wasps. All non-pollinating wasps oviposit from outside the syconium; only pollinators enter the syconium to pollinate and oviposit. Arrows signify consumer–resource interactions (in this case, prey–predator relationships) with their directions indicating energy flows. The parasitoid *Apocrypta westwoodi* is known to parasitise *Sycophaga stratheni* and *Sycophaga testacea* (P. Yadav, personal observation), whereas *Apocrypta* sp. 2 is known to parasitise *S. stratheni*, *S. testacea*, *S. fusca* (P. Yadav, personal observation) and may also parasitise pollinators. The parasitoid *Sycophaga agragensis* is a confirmed parasite of pollinators (Krishnan and Borges 2014), though its ability to use the other wasp species as hosts is not yet known. Note that *Sycophaga* is synonymised with *Apocryptophagus* (Cruaud et al. 2011). **b** Schematic diagram of syconium development phases and fig wasp oviposition periods (dashed lines). Each labelled grey box represents

the relative duration of that phase (A–E phases) on an individual representative tree. Syconium development phases consist of A pre-floral phase (male and female flowers undeveloped), B female floral phase (female flowers developed and receptive to pollination), C interfloral phase (wasp progeny and seeds developing within syconia), D male floral phase (mature wasps mate, pollinator females collect pollen from mature anthers and leave natal syconia) and E post-floral phase (syconia ripen and attract seed dispersers). The scale bar (0.5 cm) is applicable to wasp sizes. The gallers *S. stratheni* (G0) and *S. testacea* (G1) oviposit first in A-phase, followed by the galler *S. fusca* (G2) and pollinator *C. fusciceps* in B-phase. The parasitoid *Apocrypta* sp. 2 (P1) had an oviposition period spanning early- to mid-C phase, whereas *Apocrypta westwoodi* (P2) and *S. agragensis* (P3) oviposited in mid-C-phase. **c** Schematic representation of possible relationships likely to occur between *F. racemosa* fig wasp fauna. Solid and dashed lines specify direct and indirect relationships, respectively, and arrows indicate impacted syconium inhabitant. The text beside each arrow indicates the type of relationship expected between the two syconium inhabitants



Fig. 2 Wasps ovipositing in *Ficus racemosa* syconia. Crowding within and on the surface of syconia can lead to high levels of intraspecific competition that could reduce wasp reproduction. **a** Crowding within the lumen of a B-phase syconium by ovipositing

pollinator wasps (*Ceratosolen fusciceps*). **b** Crowding on the surface of a late A-phase syconium by ovipositing parasitic galler wasps (*Sycophaga fusca*). **c** Crowding on the surface of a C-phase syconium by ovipositing parasitoids (*Apocrypta* sp. 2)

it would depend on the ratio between ovule availability (number of ovules) and pollinator egg availability (based on numbers of pollinators entering the syconium) (Anstett et al. 1996; Wang et al. 2008). The relationship between seeds and galler (G1 + G2) progeny is expected to be negative (Fig. 3a) since they compete for development space and resources.

Feedback interactions of trophic level I (the plant) with trophic levels II (herbivores) and III (parasitoids)

Variation in wasp identity and frequency within syconia affects syconium development times differentially (Krishnan and Borges 2014). We used the standard deviation of frequency (SDF) of that species or species group within individual syconia to capture the variation in abundance of syconium inhabitants between syconia of a tree (Fig. 3a, b). This intra-crop variation in abundance of syconium inhabitants results in variable syconial development time (Krishnan and Borges 2014) that further affects within-tree reproductive phenology by increasing within-tree asynchrony. The SDF of gallers (G1 + G2) compared to that of other syconium inhabitants was expected to have the most effect on within-tree asynchrony (Fig. 3a). This is because gallers affect syconium development times (by causing shortening of syconium development) much more than other syconium inhabitants (Krishnan and Borges 2014). We therefore predicted that the SDF of gallers (G1 + G2) would have a positive effect on within-tree asynchrony (Fig. 3a).

Interactions within and between trophic levels II (herbivores) and III (parasitoids)

Within trophic level II, the number of galler (G1 + G2) progeny was expected to be negatively correlated with

the number of pollinator progeny (Fig. 3a) due to: (1) competition for development space and resources; and (2) exploitative or apparent competition (Fig. 1c). Interactions between trophic levels II and III were mostly expected to be predator–prey (host–parasite) relationships (Fig. 1a, c). Prey species could positively affect parasitoid abundances, whereas parasitoids could affect host prey abundances negatively (Fig. 3a). Although such systems are often non-linear, basic data exploration using scatter plots indicates that predator–prey (host–parasite) pairs in our system exhibited linear relationships (Appendix B).

Path analysis

We employed path analysis to examine interactions because it allows non-independent explanatory variables (Krishnan and Borges 2014). Furthermore, although the use of path analysis to examine feedbacks in community ecology is limited (Wootton 1994; Grace et al. 2012), it is capable of incorporating feedback loops (Hayduk 1987; Loehlin 2013). All mention of fig wasps in the analyses refers to the numbers of fully developed and enclosed offspring of the different species. Path analyses were conducted using the software LISREL 9.1 (Jöreskog and Sörbom 2012). Although variables were log-transformed to improve normality, multivariate normality was not achieved. Therefore, robust maximum likelihood (RML) estimation was used to fit structural equation models to the transformed data. Relationships between any two variables were defined as total effects partitioned into direct effects and indirect effects and are represented as standardised path coefficients (Wright 1934). Details of the methodologies followed to obtain best-fit and most parsimonious models, along with the unexplained variances of variables, are provided in Appendix C.

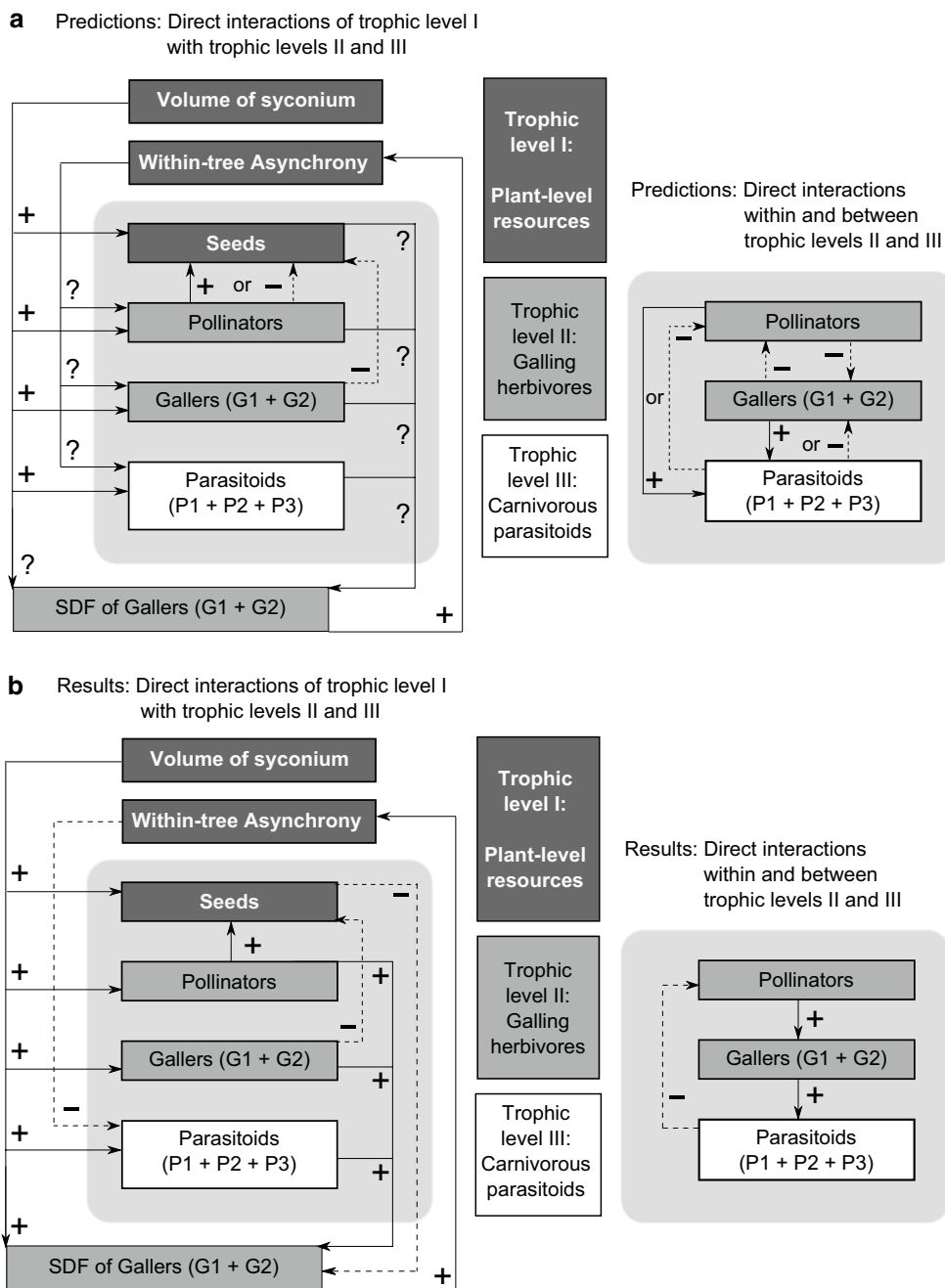


Fig. 3 Path diagrams representing predictions and results of path modelling analysis depicting the direction and sign of direct interactions between various factors. *Black, grey and white boxes* represent plant, herbivore and parasitoid trophic levels, respectively. Intra-crop variations in syconium inhabitants are represented by the standard deviation in frequency (*SDF*) of syconium contents of pollinators/seeds/gallers ($G1 + G2$)/parasitoids ($P1 + P2 + P3$) among syconia in a single reproductive episode per tree. For clarity, all relationships between trophic level I and higher trophic levels are depicted on the extreme *left*, whereas relationships within and between trophic levels II and III are depicted on the extreme *right*. The relationships between trophic level I and higher trophic levels also include the

feedback loop caused by syconium inhabitants affecting syconium development time (Krishnan and Borges 2014), and hence affecting within-tree asynchrony. The *symbols* ‘+’ and ‘-’ beside *arrows* indicate that those relationships are expected to be positive or negative, respectively. *Solid arrows* indicate positive relationships and *dotted arrows* indicate negative relationships. **a** Path diagrams representing a priori predictions of the path model constructed on the basis of the relationship diagram outlined in Appendix A. A question mark ‘?’ indicates an unknown relationship (it could be positive, negative or may not exist). **b** Path diagrams representing the results of the path analysis

Results

A model best fitting the data was obtained through systematic comparisons of the a priori and alternative models (Appendix C). For ease of presentation, results have been divided in several parts (predictions in Fig. 3a, results in Fig. 3b).

Direct and indirect effects of trophic level I (plant) on wasps in trophic levels II (gallers) and III (parasitoids)

Effect of syconium volume (inflorescence size) on wasp reproduction

Syconium volume had direct and significantly positive effects on pollinator and galler (G1 + G2) progeny (Fig. 3b; Table 1). Syconium volume had a non-significant direct effect, but a significant positive indirect effect on the parasitoids (P1 + P2 + P3) (Fig. 3b; Table 1). The total effect of this factor on all syconium contents was positive and significant (Table 1).

Effect of within-tree asynchrony (phenology) on wasp reproduction

Within-tree asynchrony had no direct effect on pollinator or galler (G1 + G2) progeny, although it had a significant negative direct effect on parasitoid progeny (Fig. 3b; Table 1). Via this effect, represented by the path: within-tree asynchrony → parasitoids (P1 + P2 + P3) → pollinators (Fig. 3b), within-tree asynchrony had a very weak, but significant, positive indirect effect on pollinator progeny (Table 1). Indirect effects of within-tree asynchrony on parasitoid progeny were non-significant, such that the total effects in these relationships were similar in magnitude and significance to the direct interactions (Table 1).

Relationships between seed production and wasp reproduction

The relationship between pollinator progeny and seeds was positive and significant (Fig. 3b; Table 1), while galler (G1 + G2) progeny had a direct and significantly negative

Table 1 Magnitudes of direct, indirect and total effects as obtained by path analysis in the best-fit and most parsimonious model

Effect of	Effect on	Effect type		
		Direct	Indirect	Total
Volume of syconium	Pollinators	0.64***	-0.02, n.s.	0.62***
	Gallers (G1 + G2)	0.12*	0.1*	0.22***
	Parasitoids (P1 + P2 + P3)	0.1, n.s.	0.04*, 0	0.14*
	Seeds	0.4***	0.05, n.s.	0.45***
Within-tree asynchrony	Pollinators	N/A	0.02*	0.02*
	Gallers (G1 + G2)	N/A	N/A	N/A
	Parasitoids (P1 + P2 + P3)	-0.11*	0.01, n.s.	-0.1*
	Seeds	N/A	<0.01, n.s.	<0.01, n.s.
Pollinators	Seeds	0.13*	-0.02, n.s.	0.11*
Gallers (G1 + G2)	Seeds	-0.11*	<0.01, n.s.	-0.11*
Pollinators	SDF of gallers (G1 + G2)	0.1, n.s.	0.04, n.s.	0.14*
Gallers (G1 + G2)	SDF of gallers (G1 + G2)	0.4***	0.05**	0.45***
Parasitoids (P1 + P2 + P3)	SDF of gallers (G1 + G2)	0.2***	-0.02, n.s.	0.18***
Seeds	SDF of gallers (G1 + G2)	-0.19***	<0.01, n.s.	-0.19***
Volume of syconium	SDF of gallers (G1 + G2)	0.18***	0.09, n.s.	0.27***
SDF of gallers (G1 + G2)	Within-tree asynchrony	0.17**	N/A	0.17**
Pollinators	Gallers (G1 + G2)	0.35***	N/A	0.35***
	Parasitoids (P1 + P2 + P3)	N/A	0.03, n.s.	0.03, n.s.
	Gallers (G1 + G2)	N/A	-0.04*	-0.04*
Gallers (G1 + G2)	Parasitoids (P1 + P2 + P3)	0.2***	-0.01*	0.19***
	Pollinators	-0.12*	N/A	-0.12*
Parasitoids (P1 + P2 + P3)	Gallers (G1 + G2)	N/A	-0.02, n.s.	-0.02, n.s.

Values are represented by standardised path coefficients (ranging from -1 to +1)

N/A indicates the absence of that path/relationship between the factors

*** $P < 0.001$, ** $P < 0.01$ and >0.001 , * $P < 0.05$ and >0.01 , n.s. $p > 0.5$

effect on seeds (Fig. 3b; Table 1). Indirect effects of pollinators and galls on seeds were non-significant, such that the total effects of these factors on seed production was similar to their direct effects (Table 1). No direct interactions were expected between the number of seeds and the number of parasitoids, nor were any such interactions observed.

Feedback effects between trophic level I (plants) and wasps in the higher trophic levels II (herbivores) and III (parasitoids)

There was feedback between the tree and abundance of syconium inhabitants involving the plant trait of within-tree asynchrony and intra-crop variation in the number of galler (G1 + G2) progeny per syconium [represented by its standard deviation as SDF of galls (G1 + G2)] (Fig. 3a, b). SDF of galls had a significant positive effect on within-tree asynchrony (Fig. 3b; Table 1). SDF of galls was itself directly and significantly positively affected by syconium volume, galler and parasitoid progeny (Fig. 3b; Table 1). Seed abundance and pollinator progeny had respectively significant negative and non-significant direct effects on SDF of galls (Fig. 3b; Table 1). All indirect effects were similar to direct effects or non-significant, with the total effects of all these factors on galler SDF similar in sign and significance to the direct effects (Table 1).

Direct and indirect effects within and between wasps in trophic levels II (herbivores) and III (parasitoids)

Relationships between herbivores (pollinating and non-pollinating galls)

There was a surprising, significant direct positive effect between pollinator and galler progeny (Fig. 3b; Table 1). However, the number of galler progeny had a significant negative indirect effect on pollinator progeny abundance (Table 1) which may have resulted from exploitative or apparent competition.

Relationships between parasitoids and herbivores (pollinating and non-pollinating galls)

The number of parasitoid (P1 + P2 + P3) progeny had a significant negative direct and total effect on pollinator progeny (Fig. 3b; Table 1), whereas the reciprocal relationship was indirect and positive, though non-significant (Table 1). The progeny of galls (G1 + G2) had a strong positive direct effect and a very low, though significant, negative indirect effect leading to a significant positive total effect on parasitoid (P1 + P2 + P3) progeny (Fig. 3b; Table 1). The negative indirect effect of galler progeny

number on parasitoid progeny abundance was likely mediated by within-tree asynchrony via the following path: galls (G1 + G2) → SDF of Galls (G1 + G2) → within-tree asynchrony → parasitoids (P1 + P2 + P3) (Fig. 3b).

Discussion

A holistic approach to plant–herbivore–parasitoid community ecology should incorporate the role of plants in mediating herbivore–parasitoid interactions, as well as examine how parasitoids could affect plant–herbivore interactions (Price et al. 1980). Our study reinforces this view by demonstrating how all three trophic levels interact within syconial microcosms. Plant trait-mediated interaction modifications were clearly traceable along linked interaction chains (Figs. 3b, 4a, b), while non-trophic phenomena such as indirect apparent/exploitative competition and facilitation are suggested based on the pattern of results obtained (Fig. 4).

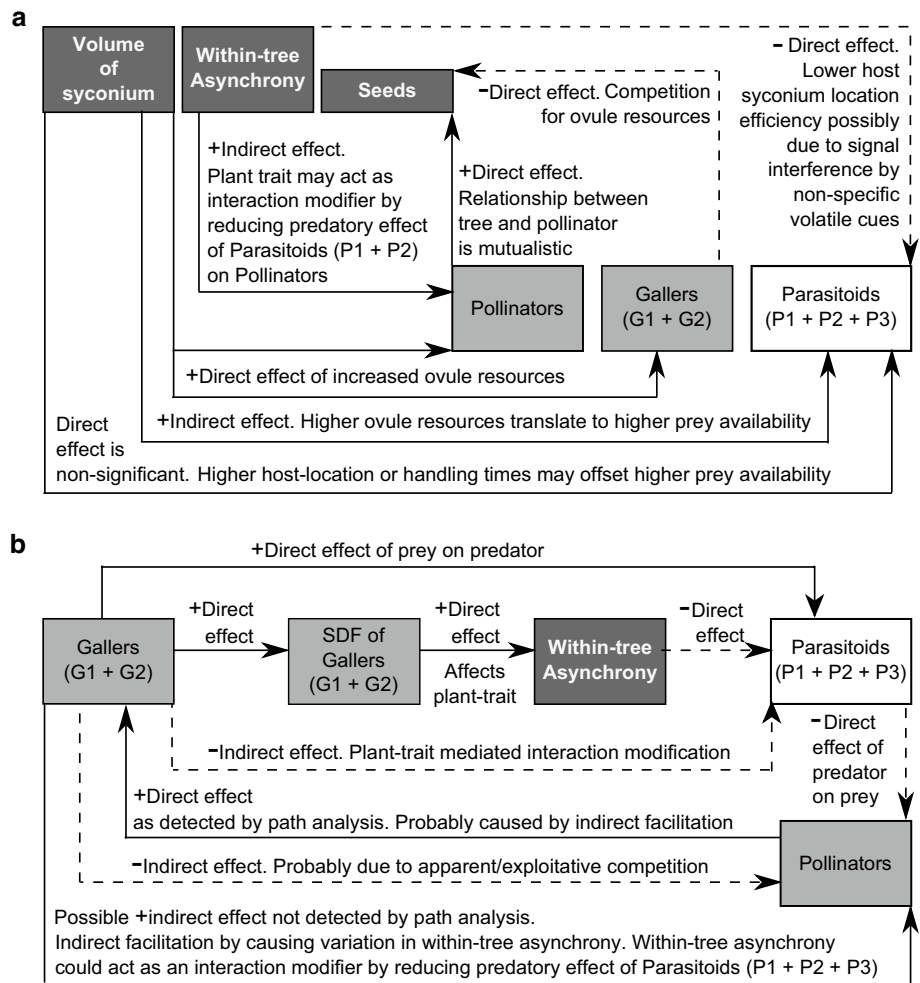
Direct and indirect interactions of trophic level I (plants) with wasps in trophic levels II (herbivore) and III (parasitoid)

Floral traits affect resources available to insects and are hence important modulators of plant interactions with mutualists and non-mutualists (Adler and Bronstein 2004; Chamberlain and Rudgers 2012). Our study indicates that floral traits (inflorescence size and phenology) not only affect wasp reproduction but also affect trophic and non-trophic interactions between the plant, its herbivores, and their parasitoids. Since the relationships between seed production and pollinator and galler (G1 + G2) reproduction were as predicted and have been relatively well studied (Anstett et al. 1996; Dunn et al. 2008; Wang et al. 2008; Al-Beidh et al. 2012), we limit our discussion to the effects of inflorescence size and phenology on wasp reproduction.

Effect of syconium volume (inflorescence size) on wasp reproduction

Larger syconia with higher volumes contain more ovules (Krishnan and Borges 2014), and represent larger resource patches to fig wasps. Predictably, syconium volume had significant positive direct effects on the abundances of most syconium inhabitants (Figs. 3b, 4a; Table 1). However, syconium volume had a surprisingly non-significant direct effect on the reproduction of the parasitoids (P1 + P2 + P3). Although larger syconia would offer more prey (which is reflected in the positive indirect effect of syconium volume on parasitoid reproduction; Table 1; Fig. 4a), they have longer flower/gall lengths (Ghara et al.

Fig. 4 Schematic representation of inferred relationships within the closed community of the *F. racemosa* microcosm based on path analysis results. *Black, grey and white boxes* represent plant, herbivore and parasitoid trophic levels, respectively. Intra-crop variation in syconium inhabitants are represented by the standard deviation in frequency (*SDF*) of syconium contents of pollinators/seeds/gallers (*G1 + G2*)/parasitoids (*P1 + P2 + P3*) among syconia in a single reproductive episode per tree. The symbols *plus* accompanied by *solid arrows* indicate positive relationships, whereas *dotted arrows* and a ‘-’ symbol indicate negative relationships. **a** The schematic represents the possible relationships between plant-associated traits and the various fig wasp fauna that could be inferred from path analysis results. **b** The schematic represents all the possible relationships between fig wasp fauna, but also includes the plant-trait of within-tree asynchrony as it plays an important role in mediating some of the relationships between the wasps



2014), which could increase the time required by these parasitoids to access prey, leaving them vulnerable to predation by ants (Ranganathan et al. 2010). Larger syconia are also likely to attract more conspecifics which will be competitors for resources. Longer host location or handling times and competition are known to reduce parasitoid reproduction (Taylor 1988; Wajnberg 2006), often leading to underutilisation of higher density patches (Walde and Murdoch 1988); since a larger syconium is a higher density patch, these phenomena may be responsible for the non-significant direct relationship between syconium volume and parasitoid reproduction (Fig. 4a).

Effect of within-tree asynchrony (phenology) on wasp reproduction

If each syconium is considered a unitary patch within the collective resource patch of the crop, a second plant trait, i.e., within-plant reproductive phenology, could affect the ability of an organism to detect the appropriate resource patch and thereby the frequency of that organism on the

patch or cluster of syconia. Host detection efficiencies in herbivorous and parasitoid insects can be lowered due to mixing and dilution or ‘interference’ of chemical cues from non-specific sources (Gols et al. 2005; Randlkofer et al. 2010). Within-plant reproductive asynchrony could increase vegetation complexity and thereby olfactory complexity (Randlkofer et al. 2010) by the presence of different phenological phases of syconia within a fig tree. Since olfactory signals mediate pollinator and non-pollinator attraction to syconia (Hossaert-McKey et al. 1994; Proffitt et al. 2007), within-tree asynchrony could affect reproduction of fig wasps by influencing their ability to detect trees bearing appropriate resource units (syconia suitable for oviposition) and thereby their numbers on (for non-pollinators) or within (for pollinators) syconia. However, within-tree asynchrony had no significant effect on the reproduction of pollinators or gallers (*G1 + G2*), though it had a significant negative direct effect on the abundances of parasitoids (*P1 + P2 + P3*) progeny (Figs. 3b, 4a, b; Table 1). The relationship of within-tree asynchrony with the number of pollinator and galler progeny could have been influenced

by a combination of other non-olfactory factors such as daily availability of oviposition sites and intra- and/or interspecific competition for these sites. Within-tree asynchrony could cause increased oviposition in individual syconia due to reduced syconium availability per day (Cook and Power 1996); the ensuing competition could decrease the number of progeny produced per syconium. Crowding by ovipositing wasps can occur both inside and outside the syconium, leading to severe competition for oviposition sites (Fig. 2). Since the overall effect of within-tree asynchrony on wasp reproduction is likely to be due to a combined effect of these factors, and the magnitude of each is unknown, it is impossible, as of now, to tease apart the contribution of each factor to the patterns observed.

While the effect of plant reproductive phenology as a trait affecting interactions between organisms utilising the same host plant is also known in several other systems (Ohgushi 2005), it is demonstrated here to our knowledge for the first time in a brood-site pollination mutualism. Within fig–fig wasp mutualisms, most studies have concentrated on elucidating the role of within-tree asynchrony on mutualism maintenance (Janzen 1979; Bronstein 1989; Gates and Nason 2012). Our study demonstrates that plant reproductive phenology affects not only mutualist reproduction but also parasite reproduction and mediates complex interactions within the system.

Feedback interactions between trophic level I (plants) and wasps in the higher trophic levels II (herbivores) and III (parasitoids)

Although positive feedback loops are hypothesised in several multitrophic communities (Craig 2010; Peterson et al. 2013), only one study, with a mutualism as the core of the community, experimentally demonstrated such feedback (Savage and Peterson 2007). Our results also suggest the existence of a positive feedback loop between a plant trait and the reproduction of a community of mutualist and non-mutualist insects. The feedback loop in this system is provided by the effect of the various syconium inhabitants on intra-crop variation in galler progeny per syconium, i.e. the SDF of galls (G1 + G2) (Fig. 3b; Table 1). Within-tree asynchrony did not affect galler reproduction directly, but did so indirectly by affecting parasitoid (P1 + P2 + P3) reproduction, which further affected the reproduction of other syconium inhabitants that ultimately influenced the SDF of galls (Fig. 3b; Table 1). Since the galls (G1 + G2) have oviposition periods in the early stages of syconium development (Ranganathan et al. 2010) and large effects on syconium development time (Krishnan and Borges 2014), the positive effect of SDF of galls (G1 + G2) on within-tree asynchrony (Fig. 3b; Table 1) was unsurprising.

The role of plant reproductive phenology in modulating interactions within and between higher trophic levels

Our analyses suggest that phenology, particularly the degree of reproductive synchrony within a tree, negatively affects the abundance of the progeny of the parasitoids (P1 + P2 + P3) found within syconia (Fig. 4a, b). Apart from its possible role as an interaction modifier in the predator–prey relationship between pollinators and parasitoids (Fig. 4a, b), within-tree asynchrony could also play a similar role in the low, but significant indirect negative effect of galler (G1 + G2) reproduction on the abundances of parasitoid (P1 + P2 + P3) progeny, and a possible indirect facilitative effect of these galls on pollinator reproduction (Fig. 4b). In this paper, we define facilitation following Callaway (2007) as an interaction within a trophic level where at least one species is positively affected provided that the two species interact locally (Verdú and Valiente-Banuet 2008).

The feedback loop between within-tree asynchrony and syconium inhabitant reproduction (Fig. 3b; Table 1) proposes some interesting herbivore-induced relationships between the various wasp inhabitants through the following path (Fig. 4b): galls (G1 + G2) → SDF of galls (G1 + G2) → within-tree asynchrony → parasitoids (P1 + P2) → pollinators. Through this path, we can see that the galler (G1 + G2) progeny abundance has a positive effect on within-tree asynchrony (Fig. 4b), which has a negative effect on the number of parasitoid (P1 + P2 + P3) progeny (Fig. 4b; Table 1), thereby leading to a negative indirect effect of galls (G1 + G2) on the reproduction of these parasitoids (Fig. 4b; Table 1). Furthermore, it is also possible that the negative indirect effect of galler (G1 + G2) progeny on parasitoid (P1 + P2 + P3) reproduction acts to lower the predatory effect of these parasitoids on pollinators, making the galls (G1 + G2) indirect facilitators of pollinator reproduction (Fig. 4b). However, this indirect facilitation was not apparent in the results (Table 1). Perhaps, by its very weak nature, it was masked by the predicted negative indirect effects of apparent and exploitative competition between galls (G1 + G2) and pollinators (Fig. 1c). Herbivore-induced effects on other herbivores, mutualists and predators are generally indirect and mediated by trait changes in plants (Price et al. 1980; Ohgushi 2005; Pagès et al. 2012), though the most commonly studied of these effects generally involve herbivore-induced nutritional or chemical changes in plant quality (Ohgushi 2005; Johnson et al. 2013). In our system, the herbivore-induced indirect effects of galls (G1 + G2) on parasitoid (P1 + P2 + P3) and pollinator reproduction is unique in that it involves the plant trait of reproductive phenology (Fig. 4b).

Indirect facilitation in trophic level II (herbivores)

The non-pollinating galls (G1 + G2) had an indirect negative effect on pollinator reproduction (Figs. 3b, 4b; Table 1), possibly due to apparent or exploitative competition (Appendix B). However, pollinator progeny had an unexpectedly direct positive effect on galler (G1 + G2) reproduction (Figs. 3b, 4b; Table 1), despite these two wasp groups being competitors for ovule resources. Unpollinated syconia infested with non-pollinating galls generally have very high abortion rates (Wang et al. 2010; A. Krishnan, personal observation), whereas syconia having non-pollinating galler and pollinator progeny have higher chances of developing without abortion. Therefore, pollinators indirectly facilitate non-pollinating galler reproduction (Fig. 4b) by ensuring survival of the shared brood-site or microcosm. The facilitator in this case, i.e., the pollinator, actually suffers negative effects due to apparent and exploitative competition with the facilitated, i.e. the galls (G1 + G2) (Fig. 4b; Table 1). This is akin to negative beneficiary feedback effects observed in plants, where facilitated plant species often compete with and have negative effects on their facilitators (Schöb et al. 2014).

Effects of trophic levels II (non-pollinating galls) and III (parasitoids) on the mutualism

The presence of exploiter or predatory satellite species in obligate pollination mutualisms could affect the reproduction of the mutualistic partners in different ways (Crabb and Pellmyr 2006; Dunn et al. 2008; Al-Beidh et al. 2012; Borges 2015). In fig–fig wasp systems, most studies indicate that the non-pollinating fig wasp fauna have negative effects mostly on pollinator reproduction, with seed production being largely unaffected (Dunn et al. 2008; Al-Beidh et al. 2012). Our results indicate that, in *F. racemosa*, the galls (G1 + G2) had a direct negative effect on the female function of the tree, i.e. seed production, as well as an indirect negative effect on the male function of the tree, i.e. pollinator reproduction (Figs. 3b, 4a, b; Table 1). Since the galls (G1 + G2) have a direct positive effect on parasitoid (P1 + P2 + P3) reproduction (Table 1; Figs. 3b, 4b), their negative effect on pollinator reproduction could be due to apparent competition. Since these galls also oviposit into syconia before and concurrently with pollinators (Fig. 1b), they are also likely to reduce pollinator reproduction through exploitative competition. A combination of these results suggests that the galls (G1 + G2) probably have a negative overall effect on the mutualism as a whole. The parasitoids (P1 + P2 + P3) have direct negative effects on pollinator reproduction (Figs. 3b, 4b; Table 1), which may be viewed as a negative effect on the mutualism, as this has a negative effect on the male function of the

tree. However, several studies suggest that parasitoids have a positive stabilising effect on the mutualism by protecting against ovule overexploitation by pollinators since they limit pollinator reproduction in outer ovules (Dunn et al. 2008; Al-Beidh et al. 2012). Whether parasitoids have net positive or net negative benefits on the fig–fig wasp system in general is as yet unresolved.

The tritrophic fig community interaction web

Analysis of interaction webs can contribute much to our understanding of the structure and diversity of tritrophic systems. Using figs and fig wasps as a model, this study demonstrates the variety of relationships that can be observed in such a multi-species tritrophic community. Apart from the obvious plant–pollinator mutualism in the system which appears secure despite parasitism, observed interactions ranged from direct predator–prey relationships to indirect competition and facilitation relationships. Several indirect relationships, however, involved all three trophic levels since these effects were mediated by plant traits. Our results not only highlight the necessity of investigating the role played by plant traits in the community ecology of tritrophic systems but also indicate that a thorough investigation of direct, indirect, trophic and non-trophic interactions is necessary to evaluate the net relationship between species. For example, that parasitic galls can be indirect facilitators of pollinator reproduction by influencing plant reproductive phenology and thereby parasitoid abundance suggests novel ways in which a mutualism can be stabilised in the face of parasitism other than conventional direct trophic relationships. Our study also suggests that trophic and non-trophic relationships in an interaction web leave signatures that are detectable even without experimental manipulation, though experiments are required to further substantiate the patterns observed. In conclusion, we believe that studies like ours will contribute to community ecology by revealing relationship diversity in mutualism-centred systems.

Author contribution statement AK and RMB conceived and designed the study. AK, MG, SK, GKP and SR collected data. AK and RMB analysed the data and wrote the paper.

Acknowledgments We thank R. Yettiraj, Pratibha Yadav and Yuvaraj Ranganathan for help in bagging and fig collection, as well as Doyle McKey, Lawrence Harder, Carol Horvitz, Vignesh Venkateswaran, Lakshy Katariya, Joyshree Chanam and two anonymous reviewers for valuable suggestions on the manuscript. This research was funded by the Ministry of Environment, Forests & Climate Change, and the Department of Biotechnology, Government of India.

Conflict of interest The authors declare that they have no conflict of interest.

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